

Profile of Liver Function in Mice after High Dose of *Psidium guajava* Linn. Extract Treatment

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Psidium guajava Linn. is a cultivated and herbal medicines from tropical and subtropical areas that extensively consume around the world as a food and folk medicine. Recent study showed that extract of *P. guajava* increased the number platelets in thrombocytopenia mice model. The purpose of this research is to know the profile of liver function after high dose treatment of *P. guajava* extract in mice. The test of acute toxicity was based on *up and down procedure* of OECD 425. The 7 female mice was divided into 3 groups. The first group was taken as a control with distilled water treatment, the second group as a test group with 2000 mg/kg b.w of *P. guajava*. extract and the third group as a test group with 5000 mg/kg b.w of *P. guajava*. extract treatment. Blood of all mice was collected to analyze biochemical test, including ALT, AST and Total Bilirubin. We found that the observation of biochemical tests by statistical approach is no differences in levels of ALT, AST and Total Bilirubin from control and both treatment groups (pe" 0.05). The extract of *P. guajava* is safe for liver function.

Keywords : ALT, AST, Total Bilirubin, Liver, *P. guajava* Linn.

The utilization of herbal plants as medicinal product and supplements has growth tremendously over the past three decades. Many researches have adjusted drug discovery, an principle group of complementary and alternative medicine (CAM) therapy, that is from herbal remedy or botanical sources^{1,2}. *P. guajava* is a typically paramount plant that found in tropical and subtropical countries. People worldwide make guava plants as traditional treatment from the fruits, leas, barks, or roots as a source. *P. guajava* has the potential pharmacologic activities including antioxidant, anti-inflammatory, antiallergy, antimicrobial, antigenotoxic, antiplasmodial, antispasmodic, antidiabetic, cytotoxic, and cardioactive, effects that

are used in the autochthonous system of medicine for the remedy or therapy of miscellaneous human ailments, such as wound, sores, bowels problems, and cholera.^{3,4}

Previous study showed that the hepatoprotective effect of *P. guajava* extract by histopathology of the liver and the phospholipid complex analysis in paracetamol-induced hepatotoxicity animal model. The research showed activity of *P. guajava* extract better than the plain extract.⁵

Our recent studies showed that *P. guajava* extract increased the megakaryocytes amount through enhancement of stem cell factor (SCF) protein expression and followed by generating an

increase of platelet count on mice model. However, it still needs to know the toxicity of the high dose before proceed to human clinical trial.^{6,7}

Toxicology is a part of pharmacology that study the side effects of chemical or drugs in biological system. The purpose of toxicological screening for the new potential drug discovery and the addition of the therapeutic potential of living sources is very essential. The Food and Drug Administration (FDA) states that it is important to find new molecules from the natural sources, such as plants, for pharmacological activity and toxicity potential in animal models.⁸

This recent investigation will make an observation on the liver. It already well known that liver is the immense, most composite organ in the gastrointestinal tract and has critical function for metabolism include drug metabolisms. The liver incorporate with three systems, which are the biochemical hepatocytic, major hepatic and the reticuloendothelial system.⁹ Again, within this research, we would like to know profile of liver function in mice after high dose of *P. guajava* extract treatment.

MATERIALS AND METHODS

Collection of *P. guajava*

The ripe fruits of *P. guajava* were collected from Dukuh Waluh Village, Purwokerto, Central Java, Indonesia. The sample was substantiated by Laboratorium Sentral Jatinangor, Universitas Padjadjaran.

Preparation of Plant Extract

P. guajava Linn. extract were made according to a previously published method.^{6,7} Briefly, guava fruits washed rigorously, sliced thinly, then macerated with 96% ethanol solvent for 24 hours and stirring occasionally. Then do the filtering using a funnel buncher. The filtral produced from the filtration was concentrated using a rotary evaporator with a temperature of 80 °C to obtain the result of a concentrated extract and then suspended by using distilled water as needed. The concentrate of guava extract is then stored at 4 °C for use in the research process.

Animals

Female Swiss Webster (20-30 g) was procured from laboratory of Pharmacology and Therapy, Universitas Padjadjaran. The animals

were acclimatized housed for 1 week under animal laboratory conditions. The mice were fed with standardized chow and clean water for drink *ad libitum* was provided. The study has got the ethical clearance from Health Research Ethics Committee (No. 1104/UN6.C.10/PN/2017), Faculty of Medicine, Universitas Padjadjaran.

Dose of Acute Toxicity Study

Oral acute toxicity test performed as previously describe.¹⁰ Briefly, mice were divided into three groups that administered orally to different groups at the high doses of both 2000 and 5000 mg/kg b.w. The sign of toxic symptoms and mortality were observed from all subject for 14 days. The first group was designated as control group and received distilled water, the second group as treatment group and received dose 2000 mg/kg b.w of *P. guajava* extract (0,2 ml/kg b.w, p.o), and the third group as treatment group and received dose 5000 mg/kg b.w of *P. guajava* extract (0,2 ml/kg b.w, p.o). All treatments were administered just once and the subject was observed for 14 days after treatment.

Biochemical Examinations

The mice were sacrificed on the fifteen day after treatment by intracutaneous injection ketamine 5% and the 1 mL blood from cardiac puncture was collected. The blood proceed further with centrifugation to collect the serum. The activities of serum aspartate transaminase (AST), alanine aminotransferase (ALT) and the extent of total bilirubin serum were analyzed with Siemens Dimension Clinical Chemistry System.

Statistical analysis

Data were determined and analyzed using One-Way Anova. The data was parametric and have normal distribution and homogeneous variants. If necessity was not matched, the test would be replaced with alternative non-parametric; Kruskal-Wallis. Furthermore, the data analyzed with Mann Whitney test to know the differences from each group. The differences of data was significant if $P < 0.05$.

RESULTS

The purpose of this study is to know profile of liver function in mice after high dose of *P. guajava* extract treatment with analyzing serum from liver enzymes and total bilirubin. Oral acute

toxicity test by administering dose limits of *Up and Down Procedure* OECD 425 (2000 and 5000 mg / kg b.w) in *P. guajava* extract did not deliver the mice to the death. Further, the observation of biochemical tests by statistical approach; ALT, AST and Total Bilirubin.

Data was collected from examination of biochemical tests and total bilirubin and showed in Table 1 and 2. We proceed the data with statistical analysis to confirm whether there were a statistically difference or not. We first analyzed

the data using the Shapiro-Wilk test and the results were not normally distributed ($p < 0.05$).

Because the data were not normally distributed, we used a non-parametric test (Kruskal Wallis Test). The result of the Kruskal Wallis test was shown to be no significant, table 1; ALT ($p = 0.029$); AST ($p = 0.029$) and table 2; Total Bilirubin ($p = 0.657$). Our present data in table 1 and 2 showed no significantly difference among control and treatment groups.

Table 1. ALT and AST Test of Liver Function

Group	ALT, U/L (Mean \pm SD)	p value	AST, U/L (Mean \pm SD)	p value
Group 1	71.50 \pm 23.33	0.267	171.00 \pm 26.87	0.029
Group 2	74.00 \pm 4.24		272.50 \pm 10.60	
Group 3	57.33 \pm 11.15		130.33 \pm 14.01	

Group 1: Control, Group 2: *P. guajava* extract (2000 mg/kg b.w, p.o), Group 3 : *P. guajava* extract (5000 mg/kg b.w, p.o). All values are Mean \pm SD. $p < 0.05$

Furthermore, we determined whether the differences between the ALT, AST and Total Bilirubin results from each group were statistically significant or not using Mann Whitney test. The result of the Mann Whitney test showed in Table 3. It indicates that there were no significant difference among group 1 and 2; group 1 and 3 and group 2 and 3 for all examination of biochemical test included ALT, AST and Total Bilirubin ($p > 0.05$). Finally, our observation indicated that high dose of ethanol extract from *P. guajava* fruits did not exhibit any remarkable alteration of the hepatic function in the healthy mice.

Table 2. Total Bilirubin Test of Liver Function

Group	Total Bilirubin, mg/dl (Mean \pm SD)	p value
Group 1	0.12 \pm 0.10	0.657
Group 2	0.12 \pm 0.07	
Group 3	0.17 \pm 0.02	

Group 1: Control, Group 2: *P. guajava* Linn. extract (2000 mg/kg b.w, p.o), Group 3: *P. guajava* extract (5000 mg/kg b.w, p.o). All values are Mean \pm SD. $p < 0.05$.

Table 3. Mann Whitney Test for each group

The data of Biochemical analysis, AST (a), ALT (b) and total bilirubin (c) were analyzed further with Mann Whitney test to know the significance from each group

a. AST	
Groups	p Value
G1 G2	0.33
G1 G3	0.20
G2 G3	0.20
b. ALT	
Groups	p Value
G1 G2	1.00
G1 G3	0.40
G2 G3	0.20
c. Total Bilirubin	
Groups	p Value
G1 G2	1.00
G1 G3	0.80
G2 G3	0.40

Group 1: Control, Group 2: *P. guajava* Linn. extract (2000 mg/kg b.w, p.o), Group 3 : *P. guajava* extract (5000 mg/kg b.w, p.o).

DISCUSSION

The fruits, leaves, barks and roots of *P. guajava* extract contain alkaloid, flavonoids, tannins and saponin³. These phytochemical are known to have several biologic roles in plants, include have pharmacological actions in human such as protect the cells from toxic agent.¹¹ Various studies have showed that extract of *P. guajava* is safe for liver. Within this study, we also found that ethanol extract of *P. guajava* fruits is safe for liver function based on the observation of biochemical tests and followed by statistical analysis.

Examination of biochemical test of doses from both 2000 mg/kg b.w and 5000 mg/kg b.w of ethanol extract of *P. guajava* fruit did not affect ALT, AST and total bilirubin extents from normal mice blood. Other previous study suggested that the -augmenting effect on the hepatocellular function of the extract could be achieve by the action of the varied extract contents, especially the presence of flavonoid which have antioxidative properties that could be as a result of its radical-scavenging activity.^{12,13} Saponins from extract have a function for depressed the level of blood cholesterol, which may effects in decreasing the metabolic problem in the liver.¹⁴ Furthermore, triterpenoid have hepatoprotective effects to significantly block the LPS (lipopolysaccharide) and (d-galactosamine) d-GaIN-convinced increases in both serum alanine aminotransferase and aspartate aminotransferase extents, exhibiting the nuclear condensation, and improvement others sign for protective effect of the liver.^{15,16}

This could be due to liver damage does not occurs after high dose of *P. guajava* extract fruits treatment. Therefore, the activities of most enzymes normally detectable in blood remain normal in healthy animal subjects.¹³ Moreover, one of major liver functions is the biochemical hepatocyte system, which is responsible for the vast majority of all metabolic activities in the body, including drug metabolism as a part of xenobiotic metabolism.⁹

The limitations in this study we only focus on liver function test since the liver is the main organ in drug metabolism. It is necessary to check the function of other organs such as kidney and brain.

CONCLUSION

The animal models are induced by extract of *P. guajava* in doses 2000 and 5000 mg/kg b.w with oral feeding which were observed for 14 days did not showed significantly liver function tests (LFTs). It can be concluded that extract of *P. guajava* is safe.

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